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Factors Affecting the Recovery of Juice and Anthocyanin from Cranberries[1]

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Abstract. **Factors affecting anthocyanin recovery in juice from pressed cranberries (*Vaccinium macrocarpon* Ait.) were investigated under laboratory conditions. Anthocyanin recovery was unaffected by cultivar, total anthocyanin content, or juice yield. Variability in anthocyanin recovery was attributed to the heterogeneity of berry samples analyzed for total and juice anthocyanin and to differences in the efficiency of pigment extraction by juice liberated during pressing. Freeze-thaw treatment of cranberries increased juice yield by as much as 50% and juice anthocyanin content by as much as 15-fold. Microscopic observation of changes at the cellular level resulting from freeze-thaw treatment supported the juice yield and pigment recovery data. Anthocyanin recovery could be increased by double pressing and by tissue homogenization.**

The anthocyanins of the American cranberry are located in the exocarp portion of the berry. During juice expression, these pigments are extracted by juice liberated from the crushed berries, thereby imparting the desired color to the product. In commercial juice production, cranberries are subjected to a freeze-thaw treatment before pressing to disrupt the cellular structure of the berries, resulting in increased juice yield and greater pigment extraction. It has been suggested that the presscake (pomace) be leached with water and re-pressed to recover unextracted pigment and other soluble solids (1). Nevertheless, after a second pressing, the pomace still may retain about 40% of the cranberry anthocyanins (9).

In the course of our investigation of methods that might be used by breeders to evaluate cranberry seedlings for fruit quality, we observed substantial differences among samples in the proportion of total anthocyanins extracted by pressing (7). Accordingly, we have studied some of the factors contributing to variability in the recovery of anthocyanins in the juice from pressed cranberries. We also have investigated some of the effects of freeze-thaw treatments on cranberry structure, juice yield, and anthocyanin recovery.

Materials and Methods

Samples of cranberries representing 45 clones were obtained from the Univ. of Massachusetts Cranberry Experiment Station in East Wareham (1980 season) and from the USDA Blueberry and Cranberry Research Center in Chatsworth, N.J. (1981 season). The cranberries were sorted manually into dark- and light-colored subsamples and were stored at 3°C or frozen at −18° until required for juice expression and analysis. Frozen samples were thawed overnight at 3° before use. Cranberry juice was

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prepared by pressing 50-g portions of chopped berries, brought to room temperature and mixed with 1.7 g rice hulls (a pressing aid), at 70.3 kg/cm^2 (1000 psi) for 20 min. The juice yield was calculated from the volume recovered by pressing. The juice soluble-solids content was determined with a refractometer. The total anthocyanin content of cranberry samples was determined by a modification of Deubert's procedure (4) wherein acidified ethanolic berry extracts were analyzed spectrophotometrically at 535 nm, the visible absorption maximum with this solvent. The juice anthocyanin content was determined spectrophotometrically at 512 nm, the absorption maximum for aqueous juice dilutions at pH 1, by a modification of the pH differential method of Fuleki and Francis (6). All procedures were described in detail previously (7).

In addition, anthocyanin was determined in juice liberated by homogenizing cranberries for 2 min at high speed in a stainless steel, semimicro blending container. Ten-g aliquots of homogenate were diluted with 190 ml distilled H_2O and clarified by the addition of 5% Celite Analytical Filter Aid, followed by filtration through Whatman No. 5 paper with suction. The resulting extract was diluted further as required, adjusted to pH 1.0 with conc. HCl, and analyzed spectrophotometrically for anthocyanin.

The double-pressing method of juice preparation was simulated by soaking the presscake from a previously pressed 50-g sample of chopped cranberries in 50-ml distilled H_2O for 10 min, draining, and retaining the unabsorbed H_2O, re-pressing the soaked berries according to our standardized procedure, and combining the expressed "juice" and unabsorbed water.

Morphological studies were carried out on 'Pilgrim' cranberries. Free-hand razor-blade sections were cut perpendicular to the surface of fresh and freeze-thaw-treated berries and were examined by light microscopy to localize pigment-containing cells. Tissue segments (2 mm^3) were fixed in a solution containing 3% glutaraldehyde and 2% paraformaldehyde in 0.07 M phosphate buffer (pH 6.8) for 2 hr, rinsed, and postfixed in 1% osmium tetroxide in the same buffer. Samples were dehydrated in a graded ethanol series and embedded in Spurr's low-viscosity resin. Sections were cut at 1 μm and examined unstained by phase contrast or stained with toluidine blue and basic fuchsin.

Results and Discussion

Relationship between juice anthocyanin and total anthocyanin. The recovery of anthocyanin in the juice pressed from cranberries can be expressed as a percentage of the total anthocyanin in the berries, as is shown below:

$$\text{anthocyanin recovery (\%)} = \frac{\text{juice anthocyanin (mg/100 ml)} \times \text{juice yield (ml/100 g)}}{\text{total anthocyanin (mg/100 g)}}.$$

In studies carried out with 72 dark- and light-colored samples of 45 representative cranberry clones that had been frozen and then thawed, the anthocyanin recovery obtained with our single-pressing procedure ranged between 25.3 and 70.0%. The mean recovery was 50.8 ± 9.6% (SD), corresponding to a coefficient of variation of 18.9%. Our data gave no indication of a cultivar effect or of a difference in anthocyanin recovery between dark- and light-colored cranberry samples. The juice anthocyanin content was directly proportional to the total anthocyanin content, the correlation coefficient being 0.85 (significant at 1% level). No relationship between extraction efficiency and the pigment content was evident.

When we used a double-pressing procedure (Table 1), the combined anthocyanin recovery for the 2 pressings was about 60%. The pigment extraction data of Staples and Francis (9), obtained with a double-pressing procedure, show a range of anthocyanin recovery values between 30 and 90%, with a mean estimated to be about 60%.

Differences in anthocyanin recovery between cranberry samples may be due to several factors, one of which is the yield of juice (5). However, variation in juice yield was relatively small in our study, the mean and SD for 85 samples (45 clones) being 80.1 ± 1.0 ml/100 g, respectively, corresponding to a coefficient of variation of only 1.2%. Francis and Servadio (5) reported a juice yield of about 70 ml/100 g when 0.454 kg (1-lb.) samples of freeze-thaw-treated cranberries were pressed for 10 min at 703 kg/cm^2 (10,000 psi) with a Carver press. Chiriboga (1) obtained a yield of 68 ml/100 g (0.081 gal per lb.) when 11.3 kg (25-lb.) batches of freeze-thaw-treated cranberries were pressed at 615 kg/cm^2 (8750 psi). Our higher values of the juice yield, obtained at only 70.3 kg/cm^2 (1000 psi), result from our practice of pressing chopped berries mixed with rice hulls for a total time of 20 min.

To obtain an independent estimate of the variability of the juice preparation method (50 g cranberries, single pressing), we measured the yield of juice from 4 representative cranberry samples, each subsampled with 4–7 replications. The pooled SD for these 21 measurements was only 2.0 ml/100 g. Therefore, it is

Table 1. Recovery of anthocyanin from freeze-thaw-treated 'McFarlin' and 'Early Black' cranberries by double pressing.

Cultivars	Juice yield[z] (ml/100 g)	Juice anthocyanin[z] (mg/100 ml)	Total anthocyanin[z] (mg/100 g)	Anthocyanin recovery (%)[y]
McFarlin				
First pressing	79.9	41.5	63.6	52.1
Second pressing	92.0[x]	5.2	63.6	7.6
				Total = 59.7
Early Black				
First pressing	81.2	49.2	75.1	53.2
Second pressing	96.1[x]	4.7	75.1	6.0
				Total = 59.2

[z]Determined in triplicate with 50-g samples; calculated on basis of 100 g.
[y]Anthocyanin recovery = juice anthocyanin × juice yield/total anthocyanin.
[x]Calculated as yield from 50 ml H_2O added to presscake following first pressing of 50-g sample.

unlikely that variation in juice yield greatly affected anthocyanin recovery.

A major source of variability in the estimation of anthocyanin recovery is the determination of the anthocyanin concentration in juice pressed from cranberry samples. Spectrophotometric analyses of the replicate juice samples prepared in the yield-variability study described above gave a pooled SD for juice anthocyanin of 3.5 mg per 100 ml juice. This estimate reflects variability in the anthocyanin content of individual berries in the sample and also variation in the efficiency of pigment extraction by the juice liberated during pressing. Extraction efficiency is related more to the extent of tissue breakdown in the exocarp during freeze-thaw treatment and to the viscosity of the juice, which may be a function of soluble pectin content (8) and temperature at the time of pressing, than to juice volume (yield) which we have shown to be relatively uniform. We believe these factors to be primarily responsible for variability in the anthocyanin content of cranberry juice rather than the spectrophotometric determination *per se*, the SD of which was reported to be only 0.08 mg anthocyanin/100 ml (6).

A second major source of variability in the estimation of anthocyanin recovery is the determination of "total" anthocyanin—i.e., anthocyanin determined by exhaustive alcoholic extraction of the cranberry sample and spectrophotometric analysis of the resulting extract. Using 50-g samples, in anticipation of the small quantities of berries that might be available in breeding studies, we found the pooled SD to be 3.9 mg anthocyanin per 100 g berries. Using a comparable method and 100-g samples, Deubert (4) reported a much smaller value for the SD (1.44 mg/100 g), the SD decreasing with increasing sample weights. When replicate analyses of the same homogenate were carried out, we and Deubert both obtained SD of only 0.6 mg anthocyanin per 100 g. Consequently, we believe that berry-to-berry variation in anthocyanin content within the sample, rather than the pigment extraction or spectrophotometric procedures, is the primary source of variability in the determination of total, as well as juice, anthocyanin (both using 50-g berry samples), and in the estimation of anthocyanin recovery.

Effects of freeze-thaw treatments. The effects of subjecting cranberries to a freeze-thaw treatment prior to juice expression are clearly seen in Table 2. The treatment not only increased the yield of juice (by nearly 50% with 'Searles') but, of even greater importance, increased the anthocyanin content of the juice by as much as 15-fold. We also observed a more rapid liberation of juice from the treated cranberries during pressing. Little or no change occurred in the juice soluble-solids content which consist mainly of glucose, fructose, quinic acid, malic acid, and citric acid (3, 8).

These results indicate that the freeze-thaw treatment disrupted the cellular structure of the berries, thereby increasing juice yield. The treatment permitted the migration of anthocyanins from the exocarp into the mesocarp and endocarp during thawing (observed in sliced berries) and thus enhanced pigment extraction during pressing. The fact that anthocyanin recovery generally did not exceed 60%, even with double pressing, suggests that some pigment-bearing cells in the exocarp were refractory to the freeze-thaw treatment.

Morphological studies. Microscopic examination of fresh and freeze-thaw-treated cranberries provided further insight into the anthocyanin recovery problem. An expoxy-embedded section of fresh 'Pilgrim' (Fig. 1) shows 2 layers of pigment-bearing cells beneath the cuticle. The outermost layer consists of small, elongated epidermal cells, densely packed with pigments. The inner layer comprises larger cells that contain pigments dispersed in the cell or precipitated along the cell wall as a consequence of fixation and dehydration. In free-hand, unfixed sections (not shown), the pigments in the epidermal cells were very dark red by transmitted light. Pigments in the inner cell layer were light red and thus appeared to be more dilute.

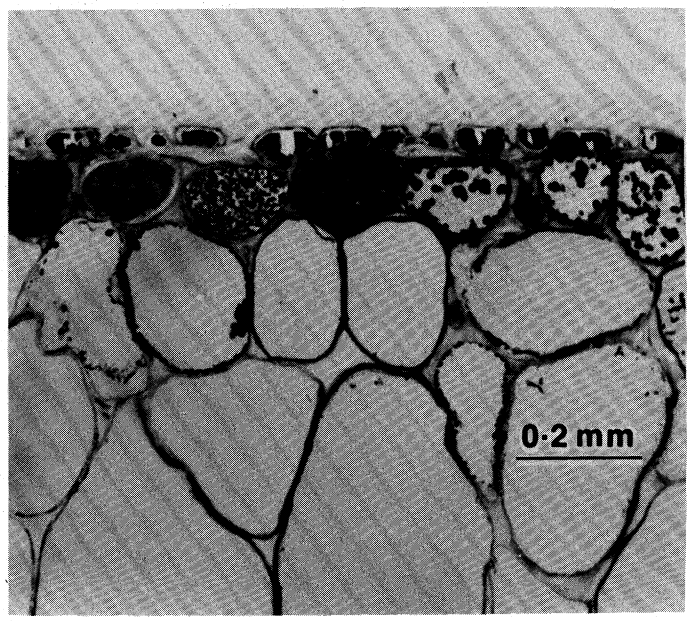

Fig. 1. Epoxy section through the skin of fresh 'Pilgrim' cranberry (80×). Two pigment-bearing cell layers beneath the cuticle are visible.

Table 2. Effects of freeze-thaw treatment on yield and composition of cranberry juice prepared by single pressing.

		Juice yield (ml/100 g)		Soluble solids (%)		Juice anthocyanin (mg/100 ml)	
Cultivar	Trial	Fresh	Freeze-thaw	Fresh	Freeze-thaw	Fresh	Freeze-thaw
Early Black	1	71	81	8.5	8.7	7.8	51.1
	2	72	81	8.6	8.8	8.7	51.2
Pilgrim	1	68	79	8.7	9.4	3.7	37.2
	2	69	80	9.1	9.8	3.5	38.5
Searles	1	54	80	10.3	10.4	1.8	34.9
	2	54	80	10.0	10.5	1.8	39.3

After the freeze-thaw treatment (Fig. 2), the outer cell layer retains its high pigment content, while the inner layer is almost devoid of pigments. Free-hand sections confirmed that thawed tissue had lost all of the brilliant red color from the inner cell layer.

We further observed that, while most of the interior cells of the cranberry were severely disrupted by the freeze-thaw treatment, the outer 3 or 4 cell layers were less damaged, presumably because of their thicker cell walls. Thus, the epidermal cells, given additional support by the cuticle, were virtually unchanged in shape after freezing and thawing, and the cells in the second pigment layer were only slightly flattened. These observations suggest that the freeze-thaw treatment enhances anthocyanin liberation from the cell layer immediately below the epidermis, but leaves a reservoir of pigments in the epidermal cells. The epidermal layer appears to be the location of the anthocyanin not recovered by pressing.

Limits of anthocyanin recovery. As much as 80–90% of the anthocyanins retained in cranberry presscake can be recovered by alcoholic extraction (2, 11). We have greatly exceeded anthocyanin recoveries attainable by pressing, without the use of alcohol extraction or freeze-thaw treatments, by homogenizing cranberries and determining the anthocyanin concentration in the liberated juice (Table 3). Anthocyanin recovery in this system may be limited by pigment degradation due to oxidation during homogenization (10), or incomplete cell disruption.

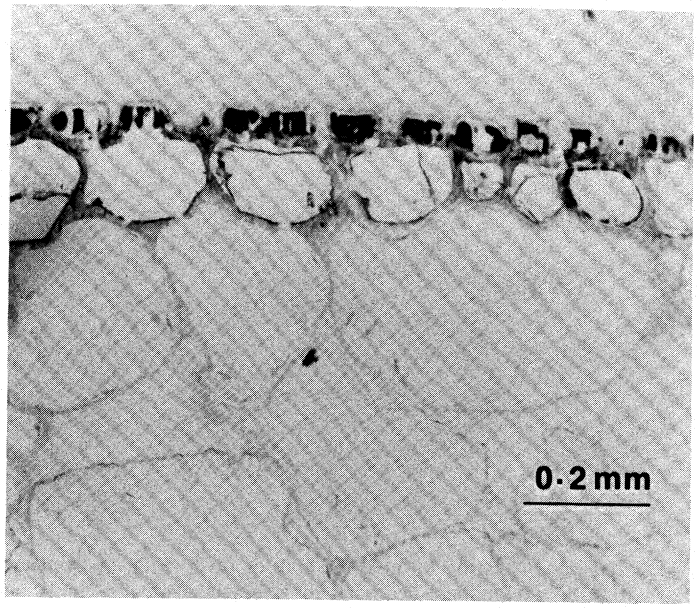

Fig. 2. Epoxy section through the skin of 'Pilgrim' cranberry given freeze-thaw treatment (80×). Pigments are retained by epidermal cells but are lost from the second layer of cells.

Table 3. Effect of freeze-thaw treatments on anthocyanin and soluble-solids content of juice from single-pressed or homogenized 'Early Black' cranberries.

Treatment[z]	Soluble solids (%)	Juice anthocyanin (mg/100 ml)	Anthocyanin recovery (%)[y]
Pressed			
Fresh	8.6	8.2	7.0
Freeze-thaw	8.8	51.2	49.2
Homogenized			
Fresh	9.7	68.8	81.9
Freeze-thaw	9.3	70.0	83.3

[z]All treatments carried out with 2 replications
[y]Based on total anthocyanin content of 84.0 mg/100 g.

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